AD_____

Award Number:
W81XWH-06-1-0688

TITLE:
Assessment of GPR30, a Seven Transmembrane-spanning Estrogen Receptor, as an Oncogene

PRINCIPAL INVESTIGATOR:
Edward Joseph Filardo, PhD

CONTRACTING ORGANIZATION:
Rhode Island Hospital
Providence, RI 02903

REPORT DATE:
October 2009

TYPE OF REPORT:
Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
17-10-2009	Final	18 Sep 2006 - 17 Sep 2009

4. TITLE AND SUBTITLE
Assessment of GPR30, a Seven Transmembrane-spanning Estrogen

Estrogen Receptor, as an Oncogene

5a. CONTRACT NUMBER
BC052936

5b. GRANT NUMBER
W81XWH-06-1-0688

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Edward Joseph Filardo, PhD

Email: edward_filardo@brown.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Rhode Island Hospital, Providence RI 02903

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland
21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT Expression of the seven transmembrane-spanning receptor (7TMR), GPR30, in primary human breast tumors is positively associated with several tumor progression variables including extra mammary metastases (Filardo et al, 2006). Altered expression of 7TMRs is linked with a spectrum of disease phenotypes, including cancer, raising the possibility that GPR30 may function as an oncogene. Prior attempts to construct transgenic mice capable of expressing (HA-GPR30) transgenes under the transcriptional control of the mouse mammary tumor virus (MMTV) promoter failed. A second no cost extension was requested to construct mice with an HA-GPR30 transgene regulated by the whey acidic protein(WAP) promoter. These mice have recently been constructed and their genotypic and phenotypic analysis is underway.

15. SUBJECT TERMS
membrane receptor, estrogen, transgenic mice

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	6	USAMRI

Table of Contents

Page

Introduction.

Breast tumor growth and survival is strongly influenced by estrogen and decisions regarding appropriate adjuvant therapy for patients with breast cancer are largely determined by the measurement of known estrogen receptors (ERs) in primary tumor biopsy specimens. However, it has long been suspected that receptors other than the known estrogen receptors may promote estrogen action. Recent findings by our lab (1-4), and others (5-8), has shown that the seven transmembrane receptor (7TMR), GPR30, promotes specific estrogen binding and biochemical signaling and in addition is linked to tumor progression in man. To further address the role of GPR30 in experimental breast tumor biology, we have proposed to generate transgenic mice capable of overexpressing wild type or mutant GPR30 using the mammary gland specific promoter, whey acidic protein.

Body.

Work conducted during second no cost extension.

The second no cost extension term of this Concept award expired on October 31, 2009. The overall goal of the concept award was to generate transgenic mice that overexpressed wild-type or active GPR30 for the purpose of assessing the biological role of this newly appreciated membrane estrogen receptor in breast cancer. We successfully generated two founder mice (T6-1A and T6-2E) that contained a stably integrated wild-type hemagluttinin (HA)- tagged GPR30 transgene during that time frame and were unable to identify an active GPR30 allele (see Nov 1, 2006- Oct 31, 2007 progress report). Accordingly, we requested and were granted, a no-cost extension to further evaluate the expression of the transgene in these mice and to determine if there was an association with the development of mammary adenocarcinoma.

The work performed during the first no cost extension was limited to Task 1 of the original *Statement of Work*. No efforts were made to pursue the development of an active GPR30 allele as remaining monies did not allow. No salary support was drawn during the no-cost extension. Monies budgeted for the generation of GPR30 CAM mice were used, in part, to conduct breeding experiments that were designed to assess MMTV-HA-GPR30 transgene expression and potential malignancy in the mammary glands of nulliparous, parous and multiparous mice.

Two lines of mice (T6-1A and T6-2E) harboring stably integrated MMTV-HA-GPR30 transgenes were generated and evaluated for transgene expression. Despite the fact that the transgene was stably inherited in both lines of mice, the MMTV-HA-GPR30 transgene protein was not detected in either lineage and was not influenced by the pregnancy status of the mice. No indications of preneoplasia or malignancy were observed in any of the transgene positive mice. However, due to the fact that we did not measure transgene expression, we are unable to conclude whether GPR30 is involved in spontaneous mammary adenocarcinoma. (see Nov 1, 2007- Oct 31, 2008 progress report

Salaries were covered entirely by Departmental Funds from Medicine at Rhode Island Hospital in both the first and second no cost extensions.

Task 1. To evaluate the impact of hyperexpressed wild-type GPR30 on mammary duct branching and predisposition for the development of invasive breast cancer.

Expression of WAP-HA-GPR30 in transgenic mouse strains.

A small amount of money $5000 remained after the first no-cost extension for us to attempt to derive new mice containing a WAP-HA-GPR30 transgene. The transgene has been produced, the mice have been generated and we are currently in the process of analyzing the phenotype.

Key Research Elements.

None.

Reportable Outcomes.

None.

Conclusions.

None yet.

.

References.

1. Filardo EJ, Quinn JA, Bland KI and AR Frackelton Jr: 2000. Estrogen-induced activation of Erk-1 and Erk-2 requires the G-protein-coupled receptor homologue, GPR30, and occurs via transactivation of the EGF receptor through release of HB-EGF. *Mol Endocrinol.* 14: 1649-1660.

2. Filardo EJ, Quinn JA, Frackelton AR Jr, Bland KI. 2002. Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. *Mol Endocrinol.* 16: 70-84.

3. Thomas P, Pang Y, Filardo EJ, Dong J. 2005. Identity of a membrane estrogen receptor coupled to a G-protein in human breast cancer cells. *Endocrinol.* 146:624-632.

4. Filardo EJ, Graeber CT, Quinn, JA, Resnick, MB, Giri D, DeLellis, RA, Steinhoff, MA, Sabo E. 2006. Distribution of GPR30, a seven-membrane-spanning estrogen receptor, in primary breast cancer and its association with clinicopathological determinants of tumor progression. *Clin Cancer Res.* 12: 6359-66.

5. Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. 2005. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science. 307:1625-1630.

6. Albanito L, Madeo A, Lappano R, Vivacqua A, Rago V, Carpino A, Oprea Tl, Prossnitz ER, Musti AM, Ando S, Maggliolini M. 2007. G coupl ed receptor 30 (GPR30) m ediates gene expression changes and growth response to 17 β-estradiol and selective GPR30 lig and G-1 in ovarian cancer cells. *Cancer Res*. 67: 1859-1866

7. Vivacqua A, Bonofiglio D, Recchia AG, Musti AM, Picard D, Ando S, Maggiolini M. 2006. The G protein-coupled recep tor GPR30 m ediates the proliferative effects induced by 17 β-estradiol and hydroxytamoxifen in endometrial cancer cells. *Mol Endocrinol.* 20:631-646

8. Smith HO, Leslie KK, Singh M, Qualls CR, Reva nkar CM, Joste NE, Prossn itz ER. 2007. GPR30: a novel indicator of poor survival for endometrial carcinoma. *Am J Obstet Gynecol.* 196: 386 e1-9

9. Clarke R. 1996. Animal models of breast cancer: their diversity and role in biomedical research. *Br Ca Res Tr*. 39: 1-6.

10. Kordon EC. 2008. MMTV-induced pregnancy-dependent mammary tumors: early history and new perspectives. *J Mam Gl Biol Neopl.* 13: 289-297.

Appendices.

None.